RAMA UNIVERSITY

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FACULTY OF ENGINEERING &TECHNOLOGY
DEPARTMENT OF BIOTECHNOLOGY

Protein blotting

- Protein blotting is an analytical method that involves the immobilization of proteins on membranes before detection using monoclonal or polyclonal antibodies. There are different blotting protocols (dot blot, 2D blot); one of the most powerful is western blotting.

Principle of Western Blotting

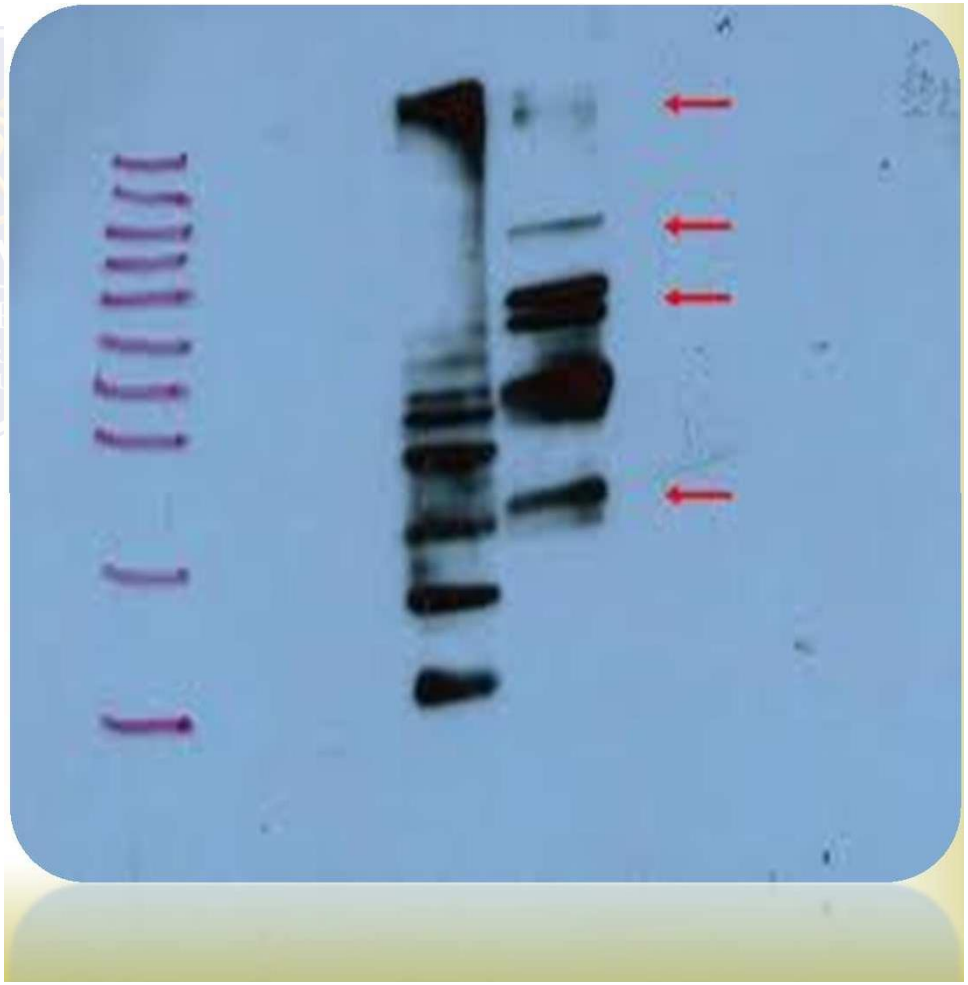
- Western blotting is an Immunoblotting technique which rely on the specificity of binding between a molecule of interest and a probe to allow detection of the molecule of interest in a mixture of many other similar molecules.
- In Western blotting, the molecule of interest is a protein and the probe is typically an antibody raised against that particular protein.
- The SDS PAGE technique is a prerequisite for Western blotting .

Western blot (Immunoblotting)

- a A technique for detecting specific proteins separated by electrophoresis by use of labeled antibodies. So called since it has some similarity to a Southern blot.

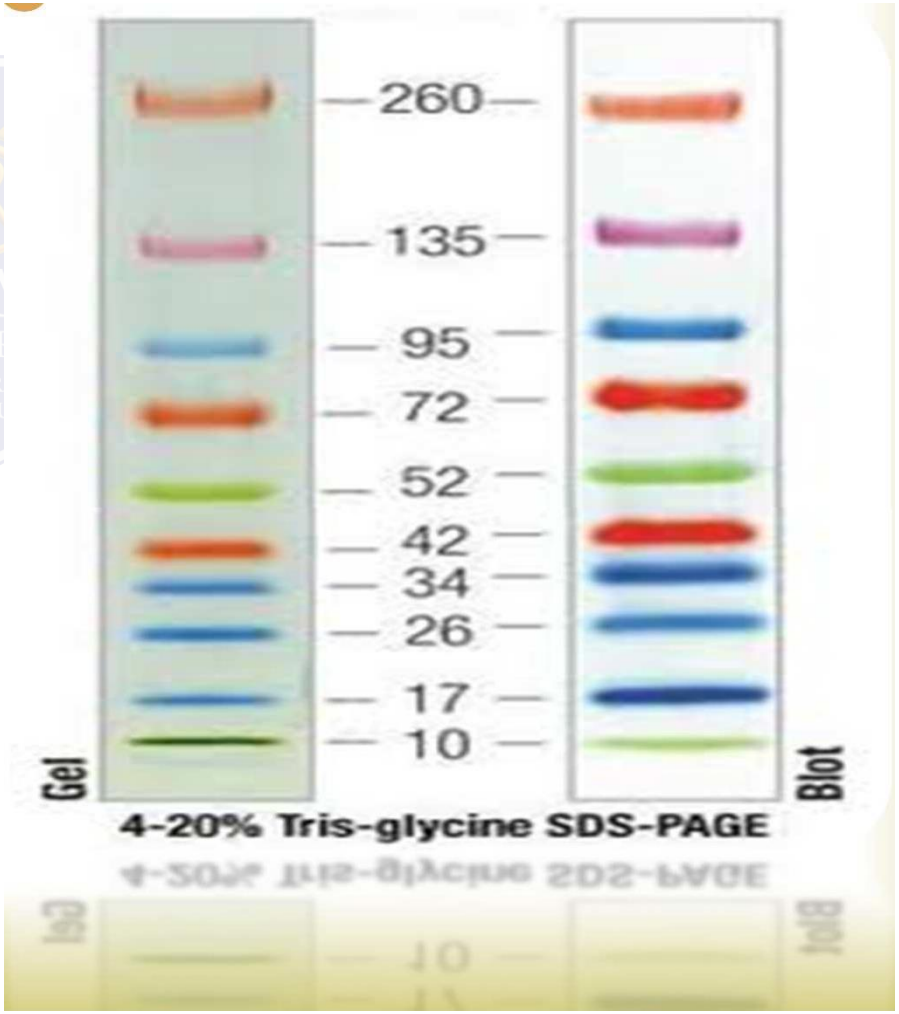
Definition

- The Western Blot is an analytical technique used to detect specific proteins in a given sample of tissue homogenate or extract.



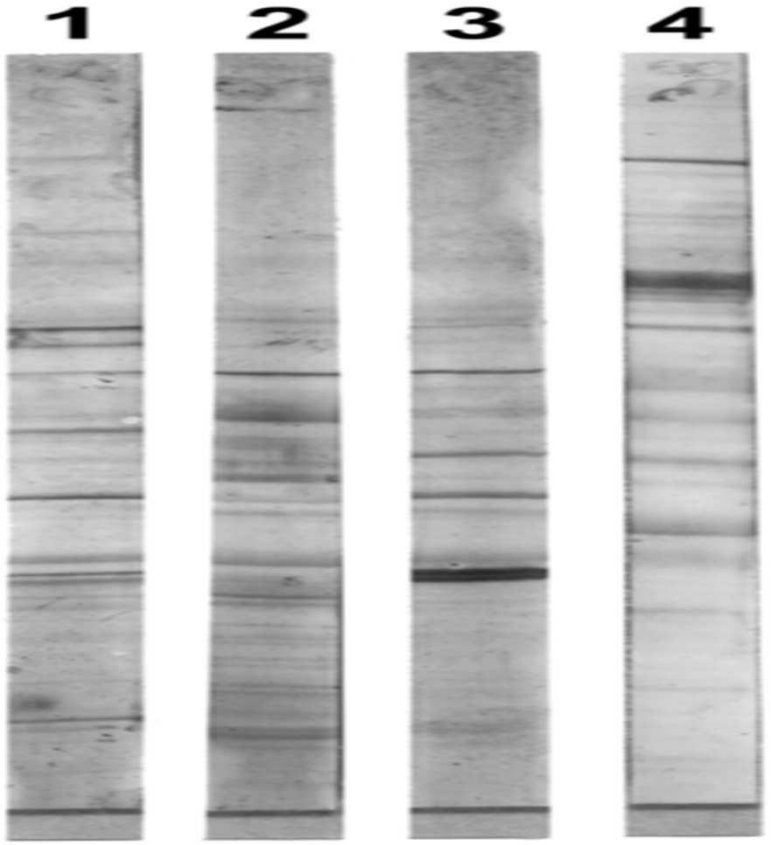
Advantages of Western Blot

- Western blot analysis can analyze any protein sample whether from cells or tissues, but also can analyze recombinant proteins synthesized in vitro.
- Western blot is dependent on the quality of antibody you use to probe for your protein of interest, and how specific it is for this protein



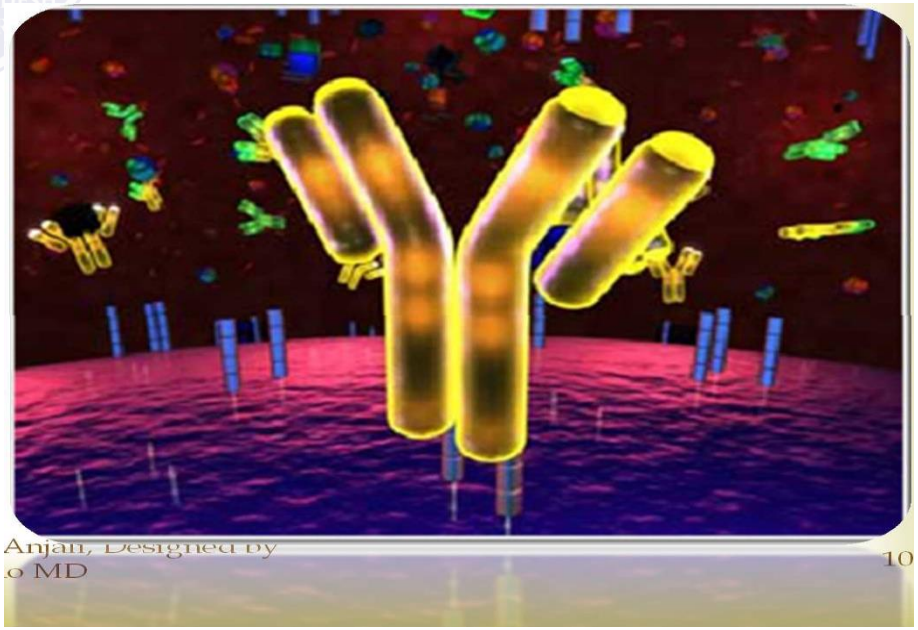
Gel electrophoresis

- a Uses gel electrophoresis to separate nativ
length of the polypeptide (denaturing conditi
the protein (native/ non-denaturing condition
transferred to a membrane (typically nitrocellu
probed (detected) using antibodies specific to



Monoclonal and Polyclonal Antibodies are used

- a Here are now many reagent companies that specialize in providing antibodies (both monoclonal and polyclonal antibodies) against tens of thousands of different proteins.



The procedure includes..

- Tissue preparation
- Gel electrophoresis
- Transfer
- Blocking
- Detection
- Analysis